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Dr. C. W. Jameson
National Toxicology Program
Report on Carcinogens, MD ED-14
P.O. Box 12233
Research Triangle Park, NC 27709

Dear Dr. Jameson:

I am writing in response to the announcement in the Federal Register, March 19, 1998 (Volume 63, Number 53), page 13418-13421 requesting comments regarding the potential delisting of saccharin from the NTP list of carcinogens. I am assuming that the first two items of information being requested, epidemiologic information and exposure data, will be addressed by other individuals writing to you with considerably more expertise in these areas than I have. However, I would only indicate again that the epidemiology does not support a conclusion of either a moderate or strong carcinogenic effect of saccharin in humans. Epidemiology cannot be used to conclusively exclude a potentially weak effect. To resolve this issue, mechanistic research is required to try to clarify the mechanisms of action in animal models so that they can be rationally interpreted in humans. Overwhelming evidence indicates that the mechanism of action for saccharin supports the conclusion that its carcinogenic effect is specific to rats, the effect is greater in male than in female rats, and it is a high dose phenomenon only. Thus, humans are not expected to have a carcinogenic effect from saccharin exposure, at low levels or at any exposure, even as high as that ingested by rats in the bioassays (see discussion below).

I have written previously to Dr. Lucier (November 24, 1997) regarding some of the issues that were debated during the meeting in Research Triangle Park on October 31, 1997. A copy of that letter is enclosed.

The other two areas for which you are requesting information in the Federal Register notice concerns mechanistic information for urinary bladder tumor formation in male rats as it relates to other test species and to humans and the adequacy of the data for tumor formation in laboratory animals at target sites other than the rat urinary bladder. I would like to discuss the latter issue first.

The very inclusion of that request indicates to me that the NTP is still being besieged with misinformation and misinterpretation regarding very old bioassays on saccharin in various animal species which have been more than adequately reviewed by numerous national and international expert panels, including the International Agency for Research on Cancer IARC) and the Office of Technology Assessment of the US Congress. All have concluded that the only credible, reproducible evidence for tumorigenicity in animals is for the urinary bladder of rats. The comments by individuals during the past year suggesting that tumors occur at other sites is based on review of

assays from 15 years ago or more, and has not taken into account the extensive research dealing with these issues. I would like to provide a detailed review of the types of tumors that have appeared in various studies and address the interpretation of them.

For most of the bioassays, occasional tumors other than the bladder have been identified, but they are usually at sites that are known to be frequently affected spontaneously in control animals for those species and strains, occur at incidences seen in controls, or have not been reproduced. The conclusions of the authors of these studies have actually stated that there was no significant increased incidence of tumors at these sites. I will try to address some of these specifically below.

A major source of continued confusion appears to be the interpretation of the bladder pellet implantation experiments in mice that were reported by Bryan and his colleagues (Science, 168: 1238-1240, 1970). The pellet implantation methodology has been subsequently demonstrated to be essentially an artifact of the laboratory, with the tumors being a consequence of the pellet itself and not the administered incorporated chemical. This has been described in extensive detail by a variety of investigators, most notably David Clayson (Clayson, JNCI, 52: 1685-1689, 1974; Clayson et al, Food and Chemical Toxicology, Vol. 33: 771-784, 1995), and specifically for saccharin by DeSesso (Comments in Toxicology, 3: 317-334, 1989). Jull, one of the originators of the pellet implantation technique, conclusively demonstrated in mice that the pellet itself is the carcinogenic agent (Jull, Cancer Letters, 6: 21-25, 1979). There is no question that saccharin is not the carcinogenic agent in mouse bladder in these pellet implantation studies. In addition to the issues regarding the pellet, it must be realized that the saccharin is leached from the pellet within essentially 24 hours. For it to have been the carcinogenic agent would require it to be the most potent chemical carcinogen yet known. This would be in contrast to all of the other data in animal models, including mice, indicating that it is not a carcinogen in mice. The amount of saccharin in the pellet is actually less than the amount of saccharin that appears in the urine of mice administered high doses of sodium saccharin in the diet . Despite a higher exposure following oral ingestion and despite that exposure continuing for 2 years instead of 1 day, no bladder lesions develop.

Reports in mice have clearly shown that in standard bioassays sodium saccharin is not carcinogenic. The only one of these studies that was done at standards approaching those expected for today's bioassays was that by Frederick et al. (Fundamental and Applied Toxicology, 12: 346-357, 1989). Female BALB/c mice fed sodium saccharin either alone or after prior treatment with 2-acetylaminofluorene (2-AAF) showed no evidence of any alterations in the bladder or other tissues in this experiment.

Roe et al. (Food and Cosmetic Toxicology, 8: 135-145, 1970) found no evidence of an increased incidence of any tumors. A few of the mice treated with high doses of saccharin in this experiment developed a variety of mostly benign tumors including ovarian granulosa cell tumor, liver hemangioma, lung adenoma, forestomach papilloma, and hepatoma. These tumors were present in single animals in the groups, were those that also occurred in controls, and were only seen after pre-treatment with benzopyrene. As concluded by the authors, there was no evidence of a carcinogenic effect.

The study by Homburger (Chemical Toxicology of Food, Pg. 359-373, 1978) concluded that there was no carcinogenic effect of sodium saccharin in rats or mice. In the rat, there was extensive contamination with the bladder parasite, Trichosomoides crassicauda. Even with this, there was not evidence of a carcinogenic effect in the bladder or other tissues. In mice, the only bladder tumor occurred in a male mouse that also had a bladder stone. There was one stomach tumor in a male mouse as well. The other lesions that occurred were those seen spontaneously in this strain of mice. There was a slight decrease in lung tumors and lymphomas, but the incidences were well within the range of the historical controls. There was a slight increase in the incidence of vascular tumors in one group given sodium saccharin, an incidence that was barely above the range of the historical controls. A second group of mice treated with sodium saccharin did not show this increase. Dr. Homburger and others that have subsequently reviewed this material concluded that there was no carcinogenic effect in this one generation study of rats and mice.

Theiss et al. (Cancer Research, 40: 4322-4324, 1980) showed that there was an increased incidence of pulmonary adenomas in the strain A mouse model with sodium saccharin, but only when administered after a high dose of urethane. There was no increased incidence or increased number of tumors in mice treated only with saccharin or treated with saccharin after a low dose of urethane. At the high dose of urethane, there was an increase in the number of tumors per animal, but not an increase in incidence. Confounding factors in interpretation of this study are also the fact that animals treated with some of the saccharin formulations showed a decreased weight gain, but most importantly, commercial preparations of saccharin were used which included significant amounts of filler material rather than using purified sodium saccharin. This study involved an evaluation of a model of benign tumors, and the only effect occurred when the sodium saccharin was administered after a high dose of the urethane. The implications and interpretation of this finding are difficult to address with respect to humans, particularly in the face of other studies in mice which have never shown an increased incidence of lung tumors.

The experiment by Salaman and Roe (British Journal of Cancer, 10: 363-378, 1956) evaluated the tumor initiating activity of saccharin in a mouse skin model using croton oil as the promoter. Seven of 20 saccharin treated animals developed benign skin papillomas with a total of 14 tumors in comparison with 4 of 19 controls with a total of 4 tumors. This was not statistically significant. In addition, there is no evidence from feeding studies of an effect on skin. It should be noted that in this study there was no effect on lung tumors, a tissue specifically evaluated by the investigators.

A recent publication by Prasad and Rai (Indian Journal of Experimental Biology, 24: 197-199, 1986) found an increased incidence of thyroid tumors in male and female mice. There are several difficulties in interpretation this experiment, including the extremely small numbers of animals in each group (10), and the difficulty of interpreting the potential dietary and cleanliness issues of this laboratory. This stands in striking contrast to all other studies in mice which have never observed any effects in thyroid. Given the extensiveness of the prominence of the types of lesions reported in this publication, it would be impossible to miss them in another study as they were grossly detectable enlargements in the neck of the live animal. They were visible externally.

A lifetime study in hamsters was reported by Althoff et al. (Cancer Letters, 1: 21-24, 1975) which found no carcinogenic effect whatsoever.

In rats, the only tumor that has been reproducibly detected has been bladder tumors. The report of benign tumors of the female genital tract are likely to be a reflection of spontaneous tumors. Since this subject has been repeatedly evaluated by a number of scientific bodies, evaluating a large number of bioassays, it seems clear that in the rat the only tumors produced by sodium saccharin administration are of the urinary bladder urothelium.

Some related issues in the interpretation of carcinogenesis bioassays in rats have also been raised. One involves the experiment reported by West el al. (Fundamental and Applied Toxicology, 7: 585-600, 1986). The authors attempted to statistically manipulate the data to conclude that there was an effect with acid saccharin and low doses of sodium saccharin by analyzing animals killed prior to terminal sacrifice separately from those examined at terminal sacrifice. This is an unacceptable approach to interpreting data. When taking into account total number of animals and total incidences, there was no increased incidence with acid saccharin, and the only dose at which there was an increased incidence with sodium saccharin was at 2.5% following prior treatment with N-methyl-N-nitrosourea (MNU). This experiment is difficult to interpret even given this result since they did not see a positive result at 5.0% sodium saccharin in the diet, a dose at which tumors had previously been detected after MNU pretreatment. There are other difficulties with the MNU model, as described in some detail in the publication by DeSesso referred to above. It should be noted that the so called tumor promotion studies with MNU and sodium saccharin were performed in female rats, not male rats, since the MNU has to be instilled directly into the bladder, a procedure that is extraordinarily difficult if not impossible to routinely accomplish in the male rat.

Further support that saccharin affects only the rat bladder comes from an experiment by Nakanishi et al. (JNCI, 68: 497-500, 1982) in which tumors were treated initially with either 2-AAF or N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN) followed by treatment with either phenobarbital or sodium saccharin. Animals treated with sodium saccharin developed tumors only in the bladder, despite the fact that AAF initiates cells in the liver as well as in the bladder.

In summary, as concluded by several previous national and international panels of experts, the only conclusion that can be drawn regarding the carcinogenicity of saccharin in animals is that it produces bladder cancer in rats, with no carcinogenic effect in mice or hamsters and tumors are not produced in any other organs in the rat.

With respect to mechanism, I detailed most of my thoughts in my previous letter to Dr. Lucier and I will not repeat them here. I would point out, however, some specific issues.

Based on the above discussion of the specificity to the rat urinary tract, some explanation must be found based on mechanistic understanding as to why the rat responds and other species do not. The rationale for the male rat being more susceptible than female rats and for the mouse not being susceptible to the effects of high doses of sodium saccharin were described in detail in the manuscript that I sent with my previous letter to Dr. Lucier (in press, in IARC Publication No. 147, C.C. Capen, E. Dybing, J. Rice, and J.D. Wilbourn, eds., Species Differences in Thyroid, Kidney

and Urinary Bladder Carcinogenesis). The lesions in the bladder develop because of the unique characteristic urinary response to high doses of sodium saccharin that occurs in rats. Multiple variables of rat urine are critical. If any one of these variables is not at critical levels, toxic and proliferative responses do not occur. It should be noted that the urinary concentrations of saccharin are higher in the mouse than in the rat following feeding of doses of sodium saccharin of 5% of the diet or higher,. Despite this higher urinary concentration of saccharin in mouse urine than in rats, no bladder lesions are produced in mice.

Of great importance in interpreting the animal studies with sodium saccharin for extrapolation to humans are the findings with other sodium salts that are administered at comparably high doses. These include such essential dietary ingredients or products of intermediary metabolism as the sodium salts of ascorbate, aspartate, erythorbate, glutamate, bicarbonate, chloride, succinate, and phosphate. These chemicals have not been studied as extensively as sodium saccharin, but where studied, the effects are identical to those of saccharin. Although there are quantitative differences in the potentency of these chemicals, the qualitative responses are the same. The identity of the findings include cytotoxicity of the urothelium, mild regenerative hyperplasia, tumor "promotion" following prior treatment with genotoxic carcinogens such as BBN or N-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide (FANFT), and lack of carcinogenicity in a one generation bioassay. The only remaining identity that needed to be shown was the effect on the bladder in a two generation bioassay. Enclosed is a manuscript describing the tumorigenicity of sodium ascorbate in the male rat utilizing a two generation protocol the same as that previously used for sodium saccharin. This manuscript is in press in Cancer Research. Like sodium saccharin, these other sodium salts appear to be effective only in the rat, with the response greater in males than in females, and with an effect only at the extraordinarily high doses used in these experiments, generally equimolar to 5% sodium saccharin in the diet.

In summary, there is adequate data available to explain the species and dose specificity for these sodium salts to clearly indicate that in humans, especially at the relatively low doses to which humans are exposed, no tumorigenic effect in the urinary tract can be expected. Since saccharin and these other sodium salts are nongenotoxic, there is no expectation for them to have an effect on any other tissues besides the urothelium based on data from the animal studies. In fact, it is difficult to conceive that substances, such as sodium ascorbate, sodium chloride, sodium bicarbonate, and other sodium salts which give a positive effect in the urinary tract in the rat, could be tumorigenic in human. The daily consumption of many of these other sodium salts is significantly higher than occurs for saccharin.

In summary, epidemiologic data provides no evidence of a moderate or strong carcinogenic effect following exposure to saccharin. A determination as to whether a weak carcinogenic effect is possible is dependent upon an understanding of the mechanism of action of sodium saccharin in the susceptible species, in this instance, the rat, and determining whether the mechanism can be produced in humans. There is more than sufficient understanding of the mechanism involved with these sodium salts to conclude that the carcinogenic effect is due to a nongenotoxic mechanism, that it is specific to the rat, that it occurs only at high doses, and that the response occurs to a greater extent in male rats than in female rats. Based on this research, it can be concluded that this

mechanism does not pertain to humans. Such a conclusion was reached by an expert panel convened at the International Agency for Research on Cancer in November, 1997. Based on the wealth of data available and the extent of our understanding of the mechanism involved, it is reasonable to remove saccharin from the list from the National Toxicology Program list of carcinogens.

Again, thank you for this opportunity to provide comments to your deliberations.

Sincerely yours,

Samuel M. Cohen, M.D., Ph.D.,
Professor and Chairman

SMC/dm